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Original Contributions

Indoleamine-2,3-dioxygenase elevated in tumor-initiating cells is suppressed by mitocans

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ABSTRACT

Tumor-initiating cells (TICs) often survive therapy and give rise to second-line tumors. We tested the plausibility of sphere cultures as models of TICs. Microarray data and microRNA data analysis confirmed the validity of spheres as models of TICs for breast and prostate cancer as well as mesothelioma cell lines. Microarray data analysis revealed the Trp pathway as the only pathway upregulated significantly in all types of studied TICs, with increased levels of indoleamine-2,3-dioxygenase-1 (IDO1), the rate-limiting enzyme of Trp metabolism along the kynurenine pathway. All types of TICs also expressed higher levels of the Trp uptake system consisting of CD98 and LAT1 with functional consequences. IDO1 expression was regulated via both transcriptional and posttranscriptional mechanisms, depending on the cancer type. Serial transplantation of TICs in mice resulted in gradually increased IDO1. Mitocans, represented by α-tocopheryl succinate and mitochondrially targeted vitamin E succinate (MitoVES), suppressed IDO1 in TICs. MitoVES suppressed IDO1 in TICs with functional mitochondrial complex II, involving transcriptional and posttranscriptional mechanisms. IDO1 increase and its suppression by VE analogues were replicated in TICs from primary human glioblastomas. Our work indicates that IDO1 is increased in TICs and that mitocans suppress the protein.

Despite advances in cancer research, neoplastic disease is on the rise [1], one reason being the nature of the tumor environment [2], including the existence of a subpopulation of tumor-initiating cells (TICs)[2] and their resistance to therapy [3,4]. TICs are "dormant" cells that feature a high level of "stemness" and the propensity to survive for long periods in their niche to give rise to second-line tumors/metastases [5,6]. Therefore, TICs ought to be able to escape tumor surveillance, a process that is not well understood thus far [7], albeit having been relatively well defined for "normal" (non-stem-like) cancer cells [8,9], termed as the "three E's" (elimination, equilibrium, escape) of tumor immune surveillance [10].

The mechanism for how TICs can escape tumor surveillance may be deduced from what is known about non-stem-like cancer cells [7–9]. The most important denominator of immune tolerance for tumor cells are T lymphocytes that have tumor-killing or -protecting

Abbreviations: ChIP, chromatin immunoprecipitation; CII, mitochondrial complex II; EMT, epithelial–mesenchymal transition; ESC, embryonal stem cell; G1, generation 1; GBM, glioblastoma multiforme; HSC, hematopoietic stem cell; IDO, indoleamine-2,3-dioxygenase; LEG, leading-edge gene; miRNA, microRNA; MitoVES, mitochondrially targeted vitamin E succinate; NS, nonsilencing; NSC, neuronal stem cell; RNAi, RNA interference; RNAPOL2, RNA polymerase 2; ROS, reactive oxygen species; SDHC, succinate dehydrogenase subunit C; TF, transcription factor; TIC, tumor-initiating cell; SQR, succinate quinone reductase; α-TOS, α-tocopheryl succinate; USI, ultrasound imaging

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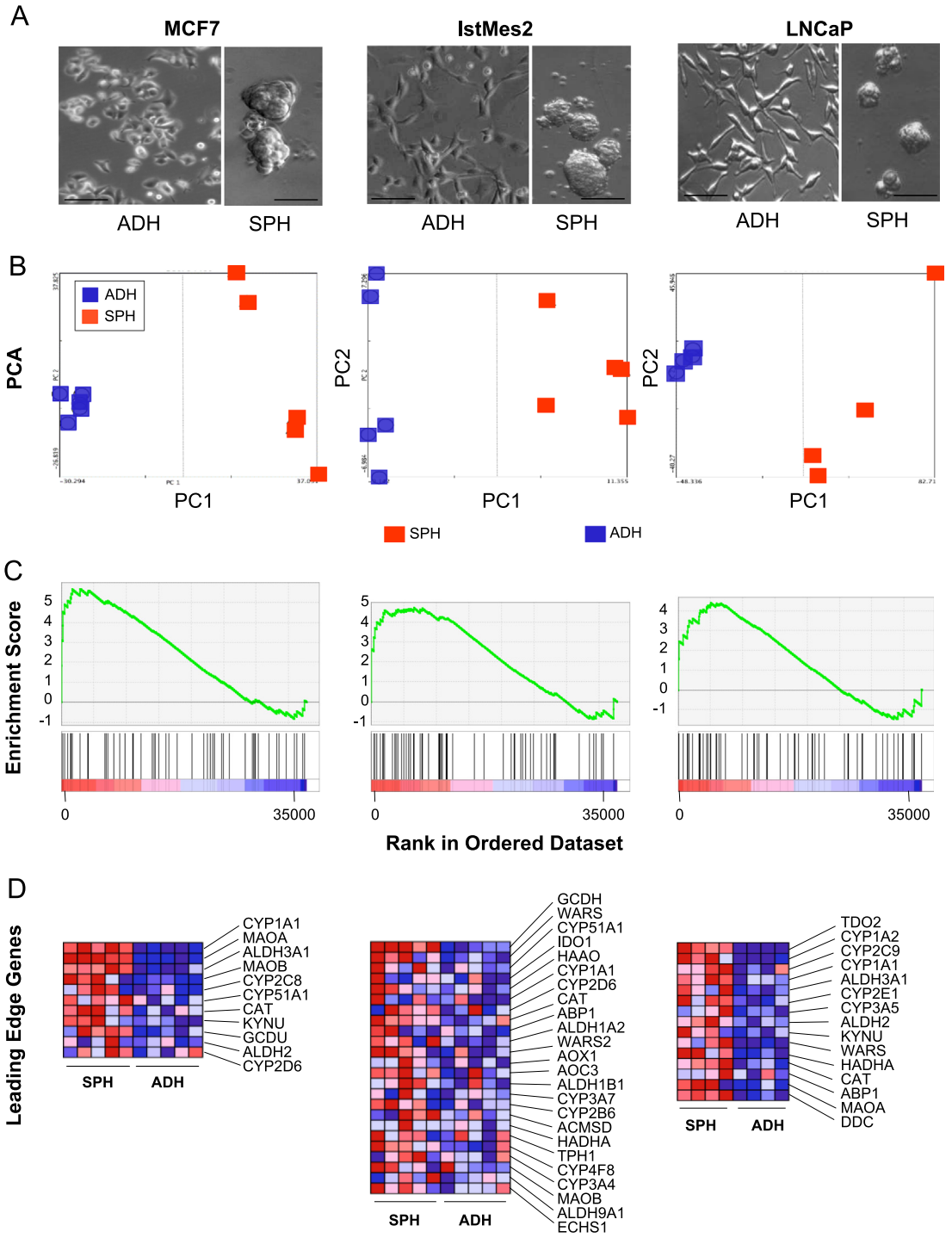

Fig. 1. Microarray analysis documents Trp metabolism increase in TICs. (A) Representative images of adherent and sphere MCF7, IstMes2, and LNCaP cells. (B) PCA of the adherent and sphere cultures of MCF7, IstMes2, and LNCaP cells. (C) GSEA for the Trp pathway in the adherent and sphere cells. (D) LEGs of the Trp pathway; red refers to high and blue to low expression. Detailed description of the analysis is under Materials and methods. Scale bar 50 micrometers (μm).

activity depending on their type in the tumor microenvironment [11,12]. One way by which cancer cells escape the immune system is to increase Trp metabolism mediated by the rate-limiting enzyme indolamine-2,3-dioxygenase (IDO) [13,14]. This results in the depletion of this essential amino acid from the tumor interstitium, which suppresses cytotoxic T cells and results in naïve T cells maturing into Treg cells [15,16]. Thus, suppression of IDO1 using small molecules has an anti-tumor effect, because it makes cancer cells more susceptible to killing by cells of the immune system [17–19].

Escape from tumor surveillance is inherent to TICs and presents a problem for therapy [20–23]. One reason is that during the development and maturation of TICs in the context of the immune system, the cells develop resistance to killing via the death receptor pathway [7,8]. It is also possible that TICs feature higher levels of Trp metabolism to minimize their vulnerability to the immune system, although this paradigm has not been well characterized thus far [23,24].

In this communication, we report that TICs derived from three different cancer cell lines and from primary glioblastoma